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Preliminary

An O-Methyltransferase (OMT) cDNA Clone in Japanese Red Pine *(Pinus densiflora)* Seedlings

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Recently, the numbers of the registered genes are rapidly growing in plant O-methyltransferases (OMTs) as many as around 50 species. Gymnosperm OMT has been discussed on lignin precursors after the last author had first characterized pine OMT[1,2]. However, the OMTs in gymnosperm are not completely understood yet in their function although an OMT cDNA of *Pinus taeda* has been characterized[1]. Because gymnosperm OMT consists of multigenes[2] and a single amino acid mutation alters the substrate specificity of a OMT[4].

A cDNA library had been constructed from the roots, which were elicited with salicylate, of Japanese red pine seedlings by using SuperScript Plasmid System (Life Technologies)[5]. Twenty-nine positive clones were obtained from 2×10^4 colonies at the first screening, and 4 single colonies were isolated at the second screening. Among them, one full-length cDNA will be described here. This cDNA was 1,772-bp long, being consisted of a 1,170-bp coding region, a 5′ untranslated region of 42 nucleotides and 3′ untranslated region of 560 nucleotides with a polyadenylated tail of 34 nucleotides. The peptide sequence deduced from the nucleotide sequence was 389 residues with relative molecular mass (Mr) of 42,683 and calculated pI values of 5.29. They had an in-frame stop codon and three possible polyadenylation signals (AATAAA). Thus the cDNA has potential to make functional OMT in Japanese red pine.

The deduced our OMT protein shows high similarity with *Pinus radiata* O-metyltransferase (GenBank Accession No. U70873) and *Pinus taeda* xylem caffeic acid *O*-methyltransferase (GenBank Accession No. U39301)[2]. Moreover, our OMT had three or five conserved sequence motifs that could possibly act as SAM-binding domains in plant SAM-dependent methyltransferases[1,3]. Plant OMTs can be classified into two distinct groups, namely Pl-OMT I and Pl-OMT II (Pl for plant)[3]. Pl-OMT I (formerly mentioned as CCoAOMTs) have 19 amino acids between Motifs A and B, and 24 amino acids between Motifs B and C. In Pl-OMT II, the distance between Motifs A and B is 52 amino acids and the one between Motifs B and C is 30 amino acids[3]. Based on this classification, our OMT belongs to Pl-OMT II.

Unlike Pl-OMT I that utilizes only CoA esters as substrates, the Pl-OMT II utilizes a various substrates such as hydroxycinnamate derivatives, chalcones and flavonols, etc. Moreover, the molecular mass of Pl-OMT II is about 40 kDa and they do not require divalent cation, i.e. Mg^{2+}, for the activity while Pl-OMT I requires the ion. The cDNA characters are in accord with our early results in a pine OMT[2].

The pine OMT cDNA was subcloned into pET-32 Xa/LIC vector to overexpress recombinant protein by using pET Trx Fusion System 32 (Novagen). The overexpressed recombinant protein was detected on SDS-PAGE while no corresponding band in the extracts from control (no plasmid or minus IPTG). The molecular size of the protein on SDS-PAGE was about 60 kDa for our OMT. This molecular size was consisted of 18 kDa of vector originated proteins and 42 kDa of the recombinant OMT, resulting 60 kDa fusion proteins.

The overexpressed proteins were also detected by Western blotting as a S·tag fusion protein. The bands recognized were the same position as the one that was observed on CBB stained SDS-PAGE gel. No corresponding band was detected from the control extracts.

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